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(71) Applicant (for all designated States except US): YEDA RESEARCH AND DEVELOPMENT CO. LTD. [IL/IL]; Weizmann Institute of Science, P.O. Box 95, 76100 Rehovot (IL).

(72) Inventors; and

(75) Inventors/Applicants (for US only): COHEN, Irun, R. [IL/IL]; 11 Hankin Street, 76354 Rehovot (IL). SHINITZKY, Meir [IL/IL]; 20 Derech Haganim, 46910 Kfar Shmaryahu (IL). MARGALIT, Raanan [IL/IL]; 76922 Ganei Yochanan (IL).

(74) Agent: BEN-AMI, Paulina; Ben-Ami & Associates, Pekeris Street 2, P.O. Box 94, 76100 Rehovot (IL).

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(54) Title: FATTY ALCOHOLS AND FATTY ACID ESTERS USEFUL FOR TREATMENT OF INFLAMMATION

(57) Abstract: Immunomodulators selected from: (a) a saturated or cis-unsaturated C₁₀ - C₂₀ fatty alcohol or an ester thereof with a C₁ - C₆ alkanolic acid; (b) a monoester of a C₂ - C₈ alkanediol or of Glycerol with a saturated or cis-unsaturated C₁₀ - C₂₀ fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated C₁₀ - C₂₀ fatty acid, are useful for treatment of inflammation, particularly immunologically-mediated inflammation such as it occurs in autoimmune diseases.

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FATTY ALCOHOLS AND FATTY ACID ESTERS USEFUL FOR TREATMENT OF INFLAMMATION

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FIELD OF THE INVENTION

The present invention relates to anti-inflammatory agents and, more particularly, to fatty alcohols, esters thereof with C₁ – C₆ alkanolic acids or esters of fatty acids with alkanediols or glycerol which are useful in the treatment of immunologically-mediated inflammation.

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Abbreviations: AA: adjuvant arthritis; CFA: complete Freund's adjuvant; EAE: experimental autoimmune encephalomyelitis; GPSCH: guinea pig spinal cord homogenate; IFA: incomplete Freund's adjuvant; OA: oleyl alcohol; PBS: phosphate-buffered saline; SC: subcutaneously.

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BACKGROUND OF THE INVENTION

Inflammation is commonly divided into three phases: acute inflammation, the immune response and chronic inflammation. Acute inflammation is the initial response to tissue injury and is mediated by the release of histamine, serotonin, bradykinin, prostaglandins and leukotrienes. The immune response, usually preceded by the acute inflammation phase, occurs when immunologically competent cells are activated in response to foreign organisms or antigenic substances liberated during the acute or chronic inflammatory response. The outcome of the immune response for the host may be beneficial, as when it causes invading organisms to be phagocytosed or neutralized. However, the outcome may be deleterious if it leads to chronic inflammation without resolution of the underlying injurious process as it occurs in rheumatoid arthritis.

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The treatment of patients with inflammation envisages the relief of pain, which is the presenting symptom and the major continuing complaint of the patient, as well as the slowing or arrest of the tissue-damaging process.

Anti-inflammatory agents are usually classified as steroidal or glucocorticoids
5 and nonsteroidal anti-inflammatory agents (NSAIDs). The glucocorticoids are powerful anti-inflammatory agents but the high toxicity associated with chronic corticosteroid therapy inhibits their use except in certain acute inflammatory conditions. Therefore, the nonsteroidal anti-inflammatory drugs have assumed a major role in the treatment of chronic conditions such as rheumatoid arthritis.

10 Among the nonsteroidal anti-inflammatory agents are included derivatives of aminoarylcarboxylic acids, arylacetic acids, arylbutyric acids, arylcarboxylic acids, arylpropionic acids, pyrazole, pyrazolone, salicylic acid and some other derivatives of different chemical structure, including specific anti-arthritic/anti-rheumatic agents.

15 Some fatty alcohols and esters of fatty acids have been described as solvents or emulsifiers for use in pharmaceutical compositions. For example, cetyl alcohol may be used in pharmaceutical compositions as emulsifying and stiffening agent (The Merck Index, pp. 347-8, # 2037), oleyl alcohol may be used as a carrier for medicaments (The Merck Index, p. 1222, # 6900), and alkyl esters of oleic acid may
20 be used as solvents for medicaments (The Merck Index, p. 6899, # 6898).

A mixture of higher aliphatic primary alcohols, primarily isolated from beeswax, was described as having moderate anti-inflammatory activity. The composition of such a mixture was not disclosed (Rodriguez et al., 1998).

Feeding laboratory animals with fish oil rich in the long-chain n-3
25 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3), was described to reduce acute and chronic inflammatory responses, to improve survival to endotoxin and in models of autoimmunity and to prolong the survival of grafted organs, and it was therefore suggested that fish oil supplementation may be clinically useful in acute and chronic
30 inflammatory conditions and following transplantation (Calder, 1998). A

pharmaceutical preparation comprising eicosapentaenoic acid and/or stearidonic acid for treatment of schizophrenia is described in WO 98/16216 and US 6,331,568.

Modified polyunsaturated fatty acids and derivatives thereof have been proposed for pharmaceutical uses. WO 99/27924 and US 6,280,755 describe anti-inflammatory fatty acids uninterrupted by a methylene group for use in topical pharmaceutical and cosmetic compositions. WO 97/38688 and US 6,262,119 describe polyunsaturated fatty acids having 1 or 2 substitutions selected from oxa and thia in position beta or gamma to the acyl group, for treating or ameliorating symptoms of T-cell mediated disease. WO 99/58122 and US 6,365,628 describe saturated fatty acids in which one or more methylene groups are substituted by O, S, SO, SO₂, or Se and alkyl esters thereof, for treatment or prevention of diabetes. US 5,019,383 describes synthetic vaccines comprising a peptide residue coupled to one or more alkyl or alkenyl groups of at least 12 carbon atoms or other lipophilic substance, wherein said alkyl or alkenyl group may be a fatty acid residue coupled to one or more functional groups of a polyfunctional group which is bound to the N-terminal amino group and/or C-terminal carboxy group of the peptide residue.

There is no description in the literature that isolated fatty alcohols or esters thereof with alkanolic acids may be used themselves as medicaments, and specifically not that they may be involved in immunomodulation of inflammation.

SUMMARY OF THE INVENTION

It has now been surprisingly found, in accordance with the present invention, that certain long-chain fatty alcohols, esters thereof with C₁ – C₆ alkanolic acids, or certain esters of long-chain fatty acids with alkanediols or glycerol can suppress inflammation in experimental adjuvant arthritis (AA) and experimental autoimmune encephalomyelitis (EAE) models in rats and can prevent graft rejection in mice.

The present invention thus relates to pharmaceutical compositions for the treatment of inflammation, particularly immunologically-mediated inflammation, comprising as active ingredient an immunomodulator selected from: (a) a saturated or cis-unsaturated C₁₀ – C₂₀ fatty alcohol or an ester thereof with a C₁ – C₆ alkanolic

acid; (b) a monoester of a $C_2 - C_8$ alkanediol or of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid.

In another embodiment, the invention relates to the use of an immunomodulator selected from: (a) a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty alcohol or an ester thereof with a $C_1 - C_6$ alkanolic acid; (b) a monoester of a $C_2 - C_8$ alkanediol or of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid, for the preparation of a pharmaceutical composition for the treatment of inflammation, in particular immunologically-mediated inflammation.

In still another embodiment, the invention relates to a method for the treatment of inflammatory disorders, in particular immunologically-mediated inflammation, which comprises administering to an individual in need thereof an effective amount of an agent selected from an immunomodulator selected from: (a) a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty alcohol or an ester thereof with a $C_1 - C_6$ alkanolic acid; (b) a monoester of a $C_2 - C_8$ alkanediol or of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the dose response effect of oleyl alcohol (OA) on adjuvant arthritis (AA). Different doses of OA were administered subcutaneously to rats once 14 days before induction of AA.

Fig. 2 is a graph showing the disease profile of Lewis rats with experimental autoimmune encephalomyelitis (EAE) and treated with oleyl alcohol. Oleyl alcohol was administered to the rats 14 days before induction of EAE. Control group was treated with incomplete Freund's adjuvant (IFA).

Fig. 3 is a graph showing the disease profile of Lewis rats with EAE and treated with IFA. IFA was administered to the rats 14 days before induction of EAE. Control group was not treated.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides immunomodulators selected from: (a) a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty alcohol or an ester thereof with a $C_1 - C_6$ alkanolic acid; (b) a monoester of a $C_2 - C_8$ alkanediol or of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid.

According to one preferred embodiment of the invention, the pharmaceutical composition comprises a long-chain saturated or unsaturated $C_{10} - C_{20}$, preferably $C_{16} - C_{20}$, most preferably a C_{18} , fatty alcohol.

Examples of $C_{10} - C_{20}$ saturated fatty alcohols that can be used according to the invention include, but are not limited to, decyl alcohol, lauryl alcohol, myristyl alcohol, stearyl alcohol and preferably cetyl alcohol (also known as palmityl alcohol).

The unsaturated fatty alcohol according to the invention has preferably one or more double bonds in the cis form and 16-18 carbon atoms and may be, without being limited to, oleyl alcohol (cis-9-octadecenol), linoleyl alcohol (cis-9,12-octadecadienol), γ -linolenyl alcohol (cis-6,9,12-octadecatrienol) and linolenyl alcohol (cis-9,12,15-octadecatrienol). In preferred embodiments, the fatty alcohol used in the compositions of the invention is cetyl, linolenyl or, most preferably, oleyl alcohol.

In another embodiment, the pharmaceutical composition of the invention comprises an ester of a fatty alcohol as defined above with a $C_1 - C_6$ alkanolic acid such as acetic acid, propionic acid, butyric acid, valeric acid and caproic acid.

In a further embodiment, the pharmaceutical composition of the invention comprises an ester of a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid with an alcohol selected from a $C_2 - C_8$ alkanediol or glycerol, said ester being a monoester with said $C_2 - C_8$ alkanediol or glycerol or a diester with glycerol.

The $C_{10} - C_{20}$ fatty acid is preferably a $C_{16} - C_{20}$, most preferably a C_{18} fatty acid. In one embodiment, the $C_{10} - C_{20}$ fatty acid is saturated such as, but without being

limited to, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and arachidic acid. In another embodiment, the C₁₀-C₂₀ fatty acid is a cis-unsaturated fatty acid such as, but without being limited to, palmitoleic acid (cis-9-hexadecenoic acid), oleic acid (cis-9-octadecenoic acid), cis-vaccenic acid (cis-11-octadecenoic acid), linoleic acid (cis-9,12-octadecadienoic acid), γ -linolenic acid (cis-6,9,12-octadecatrienoic acid), linolenic acid (cis-9,12,15-octadecatrienoic acid) and arachidonic acid (cis-5,8,11,14-eicosatetraenoic acid).

According to the invention, the alkanediol has 2 to 8, preferably 2 to 4, and more preferably, 2 carbon atoms, and is selected from, but not being limited to, 1,3-propanediol, 1,4-butanediol and, preferably, 1,2-ethylene glycol. An example of such an ester is 1,2-ethylene glycol monooleate.

According to another embodiment of the invention, the active ingredient of the pharmaceutical composition is a mono- or diester of glycerol with the long-chain fatty acid. In one preferred embodiment, the monoglyceride is glycerol monooleate. The diglycerides contain one free hydroxyl group and the other two hydroxyl groups may be both esterified with 2 molecules of the long-chain fatty acid, e.g. glycerol dioleate, or one of the hydroxyl groups is esterified with one molecule of the long-chain fatty acid and a second hydroxyl group is esterified with a C₁ – C₆ alkanolic acid such as acetic acid, propionic acid, butyric acid, valeric acid and caproic acid.

The immune system, in both its innate and adaptive arms, is involved in regulating inflammation of every type, and inflammation is a key factor in processes such as wound healing, connective tissue re-modeling, angiogenesis, organ regeneration, neuroprotection, as well as in the adaptive immune responses seen in autoimmunity, allergies, graft rejection, and infection (see Cohen, 2000; Schwartz and Cohen, 2000). Therefore, anti-inflammatory agents that modulate the inflammatory response such as those described here will be useful in a variety of conditions.

Inflammatory disorders that can be treated with the immunomodulators of the present invention include, but are not limited to, immunologically-mediated chronic or acute inflammatory disorders selected from an autoimmune disease, severe

allergies, asthma, graft rejection or for the treatment of chronic degenerative diseases such as Alzheimer's disease, and in neuroprotection, organ regeneration, chronic ulcers of the skin, and schizophrenia.

5 Examples of autoimmune diseases that can be treated according to the invention are multiple sclerosis or a human arthritic condition, e.g. rheumatoid arthritis, reactive arthritis with Reiter's syndrome, ankylosing spondylitis and other inflammations of the joints mediated by the immune system. Other autoimmune diseases are contemplated and are presented in the following list in the context of the organ or tissue involved. Thus, according to the invention, the immunologically-
10 mediated inflammatory disorder may be myasthenia gravis, Guillain-Barré syndrome, and other inflammatory diseases of the nervous system; psoriasis, pemphigus vulgaris and other diseases of the skin; systemic lupus erythematosus, glomerulonephritis and other diseases affecting the kidneys; atherosclerosis and other inflammations of the blood vessels; autoimmune hepatitis, inflammatory
15 bowel diseases, e.g. Crohn's disease, pancreatitis, and other conditions of the gastrointestinal system; type 1 diabetes mellitus (insulin-dependent diabetes mellitus or IDDM), autoimmune thyroiditis (Hashimoto's thyroiditis), and other diseases of the endocrine system.

One of the models used to test the anti-inflammatory activity of the agents
20 according to the invention is adjuvant arthritis (AA), an experimental disease of the joints inducible in some strains of rats by immunizing with *Mycobacterium tuberculosis* in complete Freund's adjuvant (CFA). These animals develop an arthritis whose features are similar to those of rheumatoid arthritis in humans and thus serve as animal models of human arthritic conditions such as rheumatoid
25 arthritis, reactive arthritis in Reiter's syndrome, ankylosing spondylitis and other inflammations of the joints which appear to be mediated by the immune system (Pearson, 1964). Adjuvant arthritis also serves as a model of immune-mediated inflammation in general including cell-mediated autoimmune reactions, graft rejection and allergic reaction. For example, treatments which can suppress
30 rheumatoid arthritis include immunosuppressive agents such as corticosteroids,

cyclosporin A (Jaffee et al., 1989; Pollock et al., 1989), azathioprine, and other immunosuppressive agents which are broadly used in the treatment of autoimmune diseases. Therefore, suppression of adjuvant arthritis by a therapeutic agent indicates that the agent is potentially useful as a broad anti-inflammatory agent.

5 The pharmaceutical composition provided by the present invention may be in solid, semisolid or liquid form and may further include pharmaceutically acceptable fillers, carriers or diluents, and other inert ingredients and excipients. The composition can be administered by any suitable route such as, but not limited to, oral, topical, or parenteral e.g. by injection through subcutaneous, intravenous, 10 intramuscular, or any other suitable route. Since many of the compounds are oily, they are preferably administered parenterally, more preferably subcutaneously. If given continuously, the compounds of the present invention are each typically administered by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. The dosage will depend of the state of the patient and 15 severity of the disease and will be determined as deemed appropriate by the practitioner.

For parenteral administration, the compounds may be formulated by mixing each at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is 20 non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. Generally, the formulations are prepared by contacting the compounds of the present invention each uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier 25 is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils can be also useful, as well as liposomes. These preparations can be made by conventional methods known to those skilled in the art, for example as described in "Remington's

Pharmaceutical Science", A.R. Gennaro, ed., 17th edition, 1985, Mack Publishing Company, Easton, PA, USA.

The invention will now be illustrated by the following non-limiting examples.

5

EXAMPLES

Example 1. Anti-inflammatory effect of oleyl alcohol and other agents-protection against adjuvant arthritis (AA)

AA was induced by immunizing inbred 8-10-week old Lewis rats (Harlan-
 10 Olac Limited, Blackthorn, Oxon, UK), at the base of the tail with 1 mg/0.1 ml of
 killed *Mycobacterium tuberculosis* (Sigma) in IFA (Sigma) as described (Pearson,
 1956). Arthritis of the limbs was noted to develop 12-14 days later and was scored
 on a scale of 0-16 summing the severity of the inflammation of each of the 4 limbs
 on a scale of 0-4, as described (Holoshitz et al., 1983). The peak of the arthritis
 15 usually was observed around day 26 after immunization.

Control rats were untreated or treated by injections of saline. A positive
 control of immunosuppression was obtained by including a group of rats treated with
 the corticosteroid agent dexamethasone (200 µg) administered intraperitoneally
 every other day beginning on day 12 after induction.

20 The immunomodulator of the invention (100 µl oleyl alcohol, glycerol mono-
 oleate, linolenyl alcohol or cetyl alcohol) was administered subcutaneously (SC)
 once 14 days before induction of AA or on day 12 after induction of AA. The
 percent inhibition of inflammation measured on the day of maximal inflammation
 was computed as follows:

$$25 \quad \frac{\text{mean maximal score of test group}}{\text{mean maximal score of control group}} \times 100\%$$

All four compounds were found to be effective, producing more than 60%
 inhibition of inflammation whereas oleic acid had no effect. The results are
 30 summarized in Table 1.

Two further experiments showed that 500 μ l of oleyl alcohol (100 μ l corresponds to about 90 mg oleyl alcohol) suppressed the inflammation by 96% and 91%.

5 **Table 1. Effects of various agents on the inflammation of adjuvant arthritis**

<u>Compound Tested</u>	<u>% Inhibition (100 μl)</u>
Glycerol mono-oleate	98%
Oleyl alcohol	78%
Linolenyl alcohol	75%
Cetyl alcohol	66%

Example 2. Protection against AA by different doses of oleyl alcohol

To study the dose response effect of oleyl alcohol in AA, oleyl alcohol was administered subcutaneously in doses of 10, 50, 100 or 500 mg to Lewis rats once 14 days before induction of AA, as described in Example 1 above.

Fig. 1 shows the dose response effect of oleyl alcohol. It can be seen that increasing doses of oleyl alcohol suppressed the arthritis. On the day of peak disease, day 26, the inflammation was suppressed by 14% (10 μ l), 61% (50 μ l), 78% (100 μ l) and 90% (500 μ l).

Example 3. Anti-inflammatory effect of oleyl alcohol and other immunomodulators and protection against EAE in DA rats

Experimental autoimmune encephalomyelitis (EAE) is an experimental autoimmune disease inducible in some strains of rats by immunization with myelin basic protein (MBP) or proteolipid protein (PLP) in complete Freund's adjuvant (CFA) or with an emulsion of the rat's spinal cord in either CFA or incomplete Freund's adjuvant (IFA). EAE in DA rats is considered as a model of chronic EAE. Within two to three weeks the animals develop cellular infiltration of the myelin sheaths of the central nervous system resulting in demyelination and paralysis. Most

of the animals die, but others have milder symptoms, and some animals develop a chronic form of the disease that resembles chronic relapsing and remitting multiple sclerosis (MS) in humans. Therefore, these animals with EAE serve as a model for the human MS autoimmune disease. EAE develops in the animal about 12 days after immunization and is characterized by paralysis of various degrees due to inflammation of the central nervous system. In some strains, like the Lewis rat, the paralysis can last up to 6-7 days and the rats usually recover unless they die during the peak of their acute paralysis. In other strains of rats like the DA rat, the paralysis can be chronic and remitting.

For the induction and clinical assessment of EAE, spinal cord obtained from DA rats is frozen, thawed and minced thoroughly with a spatula before immunization. Rats are immunized by one subcutaneous injection (just under the skin) into the dorsal base of the tail with 200 µl emulsion prepared from 1:1 IFA (Difco, Detroit, MI, USA) and antigen (volume/weight, i.e. 100 µl IFA/100 mg of whole spinal cord) or from 1:1 CFA (IFA was complemented with 4 mg/ml of *Mycobacterium tuberculosis* strain 37RA) and antigen (volume/weight, i.e. 100 µl CFA/100 mg of whole spinal cord). The emulsion was prepared by titration with a gas-tight glass syringe and a needle, 1.2 mm diameter. Rats are regularly weighed and examined for clinical signs of EAE. A four-graded scale was used to assess clinical severity: 0, no paralysis; 1, tail weakness (hanging); 2, hind limb paralysis; 3, hind and fore limb paralysis; 4, severe total paralysis (Lorentzen et al., 1995).

Groups of 5 or 7 DA strain female rats, 8-9 week old, are immunized in the hind footpads with 0.1 ml per footpad of IFA containing 100 mg of whole, homogenized DA spinal cord, for a total of 200 mg per rat. On the day of immunization, the rats are treated by SC injection with oleyl alcohol or other agent according to the invention (100 µl) or with paraffin oil (control). The rats are scored for EAE on a severity scale of 0 - 4 as described above.

Example 4. Anti-inflammatory effect of oleyl alcohol and protection against EAE in Lewis rats

EAE induced in Lewis rats is considered as a model of acute inflammation in the brain (as opposed to the chronic disease in DA rats).

5 For EAE induction, three lyophilized guinea pig spinal cord homogenate (GPSCH) emulsions were prepared as follows: (i) 25 mg of lyophilized GPSCH (GP2) was suspended in 2.5 ml of sterile PBS (Sigma) and incubated for one hour at 37° C; (ii) 54.1 mg of *Mycobacterium tuberculosis* H37Ra (MT, Difco) was suspended in 13.5 ml CFA (Sigma) containing 1mg/ml MT to obtain 5 mg/ml MT; 10 (iii) 2.5 ml CFA (5 mg/ml MT) was added into vial with 2.5 ml of PBS containing 25 mg GPSCH to yield 5 mg/ml GPSCH and 2.5 mg/ml MT. The mixture was transferred into a glass syringe connected to a second glass syringe through a Luer lock bridge. The material was mixed well by transferring from one syringe to another for about 10 minutes until the material was well emulsified. The emulsion of 15 GPSCH at a dose of 1 mg/rat and MT at a dose of 0.5 mg/rat in CFA induced EAE in rats (based on previous titration).

For the treatment, two groups of eight 9-10 weeks old Lewis rats (Harlan, Israel), were treated with the test samples (oleyl alcohol or IFA) 14 days before EAE induction. The group treated with IFA served as the control group. The test samples 20 were injected at a dose of 0.5 ml/kg once, subcutaneously. A third group of 8 rats was not treated and served as non-treated control.

EAE was induced in rats of all three groups 14 days after injection of the test samples by injection with 0.1 ml of the GPSCH emulsion in CFA into each of the hind leg foot pads (0.2 ml per rat).

25 The EAE clinical signs were observed and scored from the 9th day post-EAE induction until the termination of the experiment according to the following five-graded scale to assess clinical severity: 0, normal behavior; 1, weight loss; 2, tail weakness; 3, hind legs hypotonia and weakness; 4, hind legs paralysis; 4, severe total paralysis; 5, impaired respiration and/or convulsions and/or full paralysis or death. 30 All rats having scores of 1 and above were considered sick.

The calculation of EAE results was carried out as follows:

(i) *Calculation of the incidence of disease*

The number of sick animals in each group were summed. The incidence of disease and the % activity were calculated as follows:

$$5 \quad \text{Incidence of disease} = \frac{\text{No. of sick rats in group}}{\text{No. of rats in group}} \times 100\%$$

$$\% \text{ activity } * = 1 - \frac{(\text{disease incidence in treated group})}{\text{disease incidence in control group}} \times 100\%$$

$$10 \quad * = (\text{according to incidence})$$

(ii) *Calculation of the mean maximal score (MMS)*

The maximal score of each rat in the group were summed. The mean maximal score (MMS) and the % activity of the group were calculated as follows:

$$15 \quad \text{Mean Maximal score} = \frac{\sum \text{Maximal score of each rat}}{\text{No. of rats in the group}}$$

$$\% \text{ activity } * = \left(1 - \frac{\text{MMS of treated group}}{\text{MMS of control group}} \right) \times 100$$

$$* = (\% \text{ activity according to MMS})$$

(iii) *Calculation of the group mean score (GMS)*

20 The mean score of each rat during the observation period were summed (score 5 was counted forward). The mean score of the group and its % activity were calculated as follows:

$$\text{Mean score} = \frac{\sum \text{Group score of each rat}}{\text{No. of rats in the group}}$$

$$\% \text{ activity } * = \left(1 - \frac{\text{GMS of treated group}}{\text{GMS of control group}} \right) \times 100$$

$$25 \quad * = (\% \text{ activity according to GMS})$$

(iv) *Calculation of the mean onset of disease*

The time of disease onset (days) for each rat in the group were summed. The mean onset of disease for the group was calculated. The time of onset of disease for those rats that did not develop EAE was considered as 25 days (duration of study).

(v) *Calculation of the mean duration of disease*

The disease duration (days) of each rat in each group were summed. The mean disease duration of the group was calculated. The disease duration of rats that did not develop EAE was considered as zero.

The evaluation of the clinical manifestations of EAE, i.e. % incidence of disease, MMS, GMS, mean duration and onset of EAE disease is summarized in Table 2. The graphs of the disease profile for each group are presented in Figs. 2 and 3 for treatment with oleyl alcohol and IFA, respectively.

As shown by the results, no essential difference in incidence of disease (62.5% to 75% incidence) or mean maximum score (1.75 to 2.38 MMS) was observed between the IFA-injected groups and non-treated control group. Oleyl alcohol showed a beneficial effect on all the clinical parameters that were tested. It exhibited 77.1% activity according to group mean score (GMS) and 63% activity according to mean maximum score (MMS) compared to the non-treated control group. The mean onset of disease was 18.6 days in the oleyl alcohol treated group compared to 15.5 days in the non-treated control group. The duration of disease was 2.0 days in the oleyl alcohol treated group compared to 5.13 days in the non-treated control group. The duration of the EAE clinical signs in the tested groups was between 1 and 7 days, except one rat in the group treated with IFA. IFA had minor effect, if any, on the rat EAE. No mortality was observed in the tested groups, except one rat in the non-treated control group.

Table 2: Evaluation EAE clinical results

Group No.	Test Sample	% Incidence	% Activity Incidence	MMS	% Activity MMS	GMS	% Activity GMS	Mean Onset of disease (Day No.)	Disease duration (days)
1	OA	50.0%	33.3%	0.88	63.0%	0.22	77.1%	18.6	2.0
2	IFA	62.5%	16.7%	1.75	26.5%	0.52	45.8%	17.0	3.75
3	NTC	75.0%	NA	2.38	NA	0.96	NA	15.5	5.13

OA-Oleyl alcohol; NTC-non-treated control; NA- Not applicable

5 **Example 5. Effect of oleyl alcohol on skin allograft survival**

The immune system represents a strong barrier for successful transplantation of organs or tissues between non-genetically identical donor and recipient. Both CD4⁺ and CD8⁺ T cells participate in graft rejection.

10 Skin graft transplantation is carried out essentially as described before (Birk et al., 1999). Thus, mice are shaved and 1 cm² sections of skin are cut from the dorsal side of sacrificed donors and cleaned in PBS. Two patches of dorsal skin, 1 cm² each, are cut from the anesthetized recipients (Nembutal 6 mg/ml, 0.25 ml/mouse) in preparation for the allograft. Two donor allografts per recipient are grafted onto the dorsal lesioned patches. Histoacryl (B. Braun Melsungen AG, 15 Melsungen, Germany) is applied around the graft. Nobecutan (ASTR, Astra Tech, Glos G15, UK) is sprayed over the grafts.

In the experiment, groups of 6 BALB/c female mice, 8-week old, are grafted with 1 cm², full thickness skin grafts from C57BL/6 female mice, 8-week old. On the day of grafting, a group of recipient mice is treated either with paraffin oil or SC 20 with 100 µl oleyl alcohol or another immunomodulator according to the invention. The day of rejection is scored. The transplanted skin in the mice treated with the immunomodulator survives longer in comparison with the untreated control mice.

25 **Example 6. Prevention and treatment of SLE**

Systemic lupus erythematosus is an autoimmune disease in which both autoantibodies and immune complexes are involved. In order to test the

immunomodulators of the invention, mice with experimental SLE or (NZBxNZW)F1 mice that spontaneously develop autoimmune diseases that closely resemble SLE, can be used.

In order to induce experimental SLE, BALB/c mice are immunized with the human or murine anti-DNA monoclonal antibody 16/6Id (20 µg/mouse) in CFA in the hind footpads and boosted 3 weeks later with the same amount of the immunizing antibody in PBS. The mice are then tested for autoantibody production and clinical manifestations characteristic of experimental SLE. In order to either prevent induction of experimental SLE or to cure mice afflicted with the disease, mice are given oleyl alcohol or another immunomodulator according to the invention subcutaneously (100 µl per mouse) before or concomitant with the immunization and some weeks after immunization. The number of injections is based on the effect of the tested compound on the disease induction and progression. The animals are regularly weighed and examined for clinical signs of SLE as described, for example, in WO 96/30057.

Example 7. Prevention and treatment of autoimmune thyroiditis

Experimental autoimmune thyroiditis (EAT) can be induced in a number of animals by immunizing with thyroglobulin in CFA. Both humoral antibodies and T_{DTH} cells directed against the thyroglobulin develop, resulting in thyroid inflammation. EAT appears to best mimic Hashimoto's thyroiditis.

EAT is induced as previously described (Rose et al., 1971) by injecting each mouse subcutaneously with thyroglobulin extract obtained from one thyroid gland. The extract is emulsified in IFA (Difco Laboratories, Detroit, Mich.), to which are added 7mg/ml *Mycobacterium tuberculosis*, H37Ra strain (Difco Laboratories). This injection is repeated one week later. Donors of thyroglobulin extract are mice of the C3H/eB strain. 4-5 weeks later, EAT is assayed by removing thyroid glands of recipient mice, fixing them in 10% formalin solution and then in 70% alcohol, and examining microscopic sections stained with hematoxylin and eosin. Microscopic slides are coded and examined without knowledge of their identity. A diagnosis of

EAT is made by observing at least one unequivocal focus of infiltration by mononuclear cells. Treatment is performed by injecting SC oleyl alcohol or another immunomodulator (100 μ l per animal) before induction of EAT, concomitant with or thereafter (control animals are injected paraffin oil), and the animals are regularly
5 weighed and examined for clinical signs of EAT by known conventional methods.

REFERENCES

Birk et al., 1999. The 60kDa heat shock protein modulates allograft rejection. Proc. Nat. Acad. Science USA. 96: 5159-63.

5

Calder, PC. 1998. Immunoregulatory and anti-inflammatory effects of n-3 polyunsaturated fatty acids. Braz. J. Med. Biol. Res. 31:467-90.

10

Cohen, IR. 2000, *Tending Adam's Garden: Evolving the Cognitive Immune Self*. Academic Press, London, UK.

Cohen, IR. 2000. "Discrimination and Dialogue in the Immune System". Seminars in Immunology. 12: 215-219; 269-271; 321-323.

15

Holoshitz, Y. et al., 1983. "Lines of T lymphocytes mediate or vaccinate against autoimmune arthritis", Science 219: 56.

20

Jaffee, B.D. et al., 1989, "The effect of immunomodulating drugs on adjuvant-induced arthritis in Lewis rats", Agents Actions. 27 (3-4): 344-6.

25

Lorentzen J.C. et al., 1995, "Protracted, relapsing and demyelinating experimental autoimmune encephalomyelitis in DA rats immunized with syngeneic spinal cord and incomplete Freund's adjuvant", J. Neuroimmunology. 63: 193-205.

Pearson, CM. 1964, "Experimental models in rheumatoid disease", Arthritis Rheum. 7:80.

30

Pearson, CM. 1956, "Development of arthritis, peri arthritis and periostitis in rats given adjuvant", Proc. Soc. Exp. Biol. Med. 91:91.

Pollock et al., 1989, "Pharmacokinetic analysis of cyclosporine in adjuvant arthritic rats", *Drug Metab. Dispos.* 17 (6): 595-9.

5 Rodriguez, M.D., Gamez, R., Sanchez, M and H. Garcia. 1998, *J. Appl. Toxicol.* 18: 313-6.

Rose, N.R., F.J. Twarog and A.J. Crowe. 1971. Murine thyroiditis: importance of adjuvant and mouse strain for the induction of thyroid lesions. *J. Immunol.* 106:698)

10

Schwartz, M. and Cohen.IR. 2000, "Autoimmunity can benefit self-maintenance". *Immunol Today.* 21:265-8.

The Merck Index, 13th Edition, 2001, Merck & Co. Inc., Rahway, N.J., U.S.A.

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CLAIMS:

1. A pharmaceutical composition for treatment of inflammation, particularly immunologically-mediated inflammation, comprising as active ingredient an immunomodulator selected from: (a) a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty alcohol or an ester thereof with a $C_1 - C_6$ alkanolic acid; (b) a monoester of a $C_2 - C_8$ alkanediol or of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid.
2. The pharmaceutical composition according to claim 1, wherein the active ingredient is a saturated $C_{10} - C_{20}$ fatty alcohol.
3. The pharmaceutical composition according to claim 2, wherein the saturated $C_{10} - C_{20}$ fatty alcohol is selected from decyl alcohol, lauryl alcohol, myristyl alcohol, cetyl alcohol and stearyl alcohol.
4. The pharmaceutical composition according to claim 1, wherein the active ingredient is a cis-unsaturated $C_{16} - C_{18}$ fatty alcohol.
5. The pharmaceutical composition according to claim 4, wherein the cis-unsaturated $C_{16} - C_{18}$ fatty alcohol is selected from oleyl alcohol, linoleyl alcohol, γ -linolenyl alcohol and linolenyl alcohol.
6. The pharmaceutical composition according to claim 1, wherein the active ingredient is an ester of a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty alcohol with a $C_1 - C_6$ alkanolic acid.
7. The pharmaceutical composition according to claim 1, wherein the active ingredient is a monoester of a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid with a $C_2 - C_8$ alkanediol.

8. The pharmaceutical composition according to claim 7, wherein said alkanediol is selected from 1,2-ethylene glycol, 1,3-propanediol and 1,4-butanediol.
9. The pharmaceutical composition according to claim 1, wherein the active ingredient is a monoester of a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid with glycerol.
10. The pharmaceutical composition according to claim 1, wherein the active ingredient is a diester of a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid with glycerol.
11. The pharmaceutical composition according to any one of claims 1 and 7 to 10, wherein said fatty acid is a saturated $C_{10}-C_{20}$ fatty acid.
12. The pharmaceutical composition according to claim 11, wherein said saturated fatty acid is selected from capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and arachidic acid.
13. The pharmaceutical composition according to any one of claims 1 and 7 to 10, wherein said fatty acid is a cis-unsaturated $C_{10} - C_{20}$ fatty acid.
14. The pharmaceutical composition according to claim 13, wherein said cis-unsaturated $C_{10}-C_{20}$ fatty acid is selected from palmitoleic acid, oleic acid, cis-vaccenic acid, linoleic acid, γ -linolenic acid, linolenic acid, and arachidonic acid.
15. The pharmaceutical composition according to claim 14, wherein said active ingredient is glycerol monooleate.
16. The pharmaceutical composition according to claim 14, wherein said active ingredient is glycerol dioleate.

17. A pharmaceutical composition according to any one of claims 1 to 16 for the treatment of immunologically-mediated chronic or acute inflammatory disorders selected from an autoimmune disease, severe allergies, asthma, graft rejection or for
5 the treatment of chronic degenerative diseases such as Alzheimer's disease, and in neuroprotection, organ regeneration, chronic ulcers of the skin, and schizophrenia.

18. The pharmaceutical composition according to claim 17, wherein said autoimmune disease is multiple sclerosis or a human arthritic condition.

10 19. The pharmaceutical composition according to claim 18, wherein said human arthritic condition is selected from rheumatoid arthritis, reactive arthritis with Reiter's syndrome, ankylosing spondylitis and other inflammations of the joints mediated by the immune system.

15 20. The pharmaceutical composition according to claim 17, wherein said immunologically-mediated inflammatory disorder is myasthenia gravis, Guillain-Barré syndrome, and other inflammatory diseases of the nervous system; psoriasis, pemphigus vulgaris and other diseases of the skin; systemic lupus erythematosus,
20 glomerulonephritis and other diseases affecting the kidneys; atherosclerosis and other inflammations of the blood vessels; autoimmune hepatitis, inflammatory bowel diseases, pancreatitis, and other conditions of the gastrointestinal system; type 1 diabetes mellitus, autoimmune thyroiditis, and other diseases of the endocrine system.

25 21. Use of an immunomodulator selected from: (a) a saturated or cis-unsaturated C_{10} – C_{20} fatty alcohol or an ester thereof with a C_1 – C_6 alkanolic acid; (b) a monoester of a C_2 – C_8 alkanediol or of glycerol with a saturated or cis-unsaturated C_{10} – C_{20} fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated C_{10} – C_{20}

fatty acid, for the preparation of a pharmaceutical composition for the treatment of inflammation, particularly immunologically-mediated inflammation.

22. The use according to claim 21, wherein said immunomodulator is a saturated
5 C₁₀-C₂₀ fatty alcohol.

23. The use according to claim 22, wherein said saturated C₁₀-C₂₀ fatty alcohol is selected from decyl alcohol, lauryl alcohol, myristyl alcohol, cetyl alcohol and stearyl alcohol.

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24. The use according to claim 21, wherein said immunomodulator is a cis-unsaturated C₁₆-C₁₈ fatty alcohol.

25. The use according to claim 24, wherein the cis-unsaturated C₁₆-C₁₈ fatty alcohol
15 is selected from oleyl alcohol, linoleyl alcohol, γ -linolenyl alcohol and linolenyl alcohol.

26. The use according to claim 21, wherein the immunomodulator is an ester of a saturated or cis-unsaturated C₁₀ - C₂₀ fatty alcohol with a C₁ - C₆ alkanolic acid.

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27. The use according to claim 21, wherein said immunomodulator is a monoester of a saturated or cis-unsaturated C₁₀ - C₂₀ fatty acid with a C₂ - C₈ alkanediol.

28. The use according to claim 27, wherein said alkanediol is selected from 1,2-
25 ethylene glycol, 1,3-propanediol and 1,4-butanediol.

29. The use according to claim 21, wherein said immunomodulator is a monoester of glycerol with a saturated or cis-unsaturated C₁₀-C₂₀ fatty acid.

30. The use according to claim 21, wherein said immunomodulator is a diester of glycerol with a saturated or cis-unsaturated C₁₀-C₂₀ fatty acid.

5 31. The use according to any one of claims 21 and 27 to 30, wherein said fatty acid is a saturated C₁₀-C₂₀ fatty acid.

32. The use according to claim 31, wherein said saturated C₁₀-C₂₀ fatty acid is selected from capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and arachidic acid.

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33. The use according to any one of claims 21 and 27 to 30, wherein said fatty acid is a cis-unsaturated C₁₀-C₂₀ fatty acid.

15 34. The use according to claim 33, wherein said cis-unsaturated C₁₀-C₂₀ fatty acid is selected from palmitoleic acid, oleic acid, cis-vaccenic acid, linoleic acid, γ -linolenic acid, linolenic acid, and arachidonic acid.

35. The use according to claim 34, wherein said immunomodulator is glyceryl monooleate.

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36. The use according to claim 34, wherein said immunomodulator is glyceryl dioleate.

25 37. The use according to any one of claims 21 to 36, wherein said pharmaceutical composition is for the treatment of immunologically-mediated chronic or acute inflammatory disorders selected from an autoimmune disease, severe allergies, asthma, graft rejection or for the treatment of chronic degenerative diseases such as Alzheimer's disease, and in neuroprotection, organ regeneration, chronic ulcers of the skin, and schizophrenia.

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38. The use according to claim 37, wherein said autoimmune disease is multiple sclerosis or a human arthritic condition.

39. The use according to claim 38, wherein said human arthritic condition is selected from rheumatoid arthritis, reactive arthritis with Reiter's syndrome, ankylosing spondylitis and other inflammations of the joints mediated by the immune system.

40. The use according to claim 37, wherein said immunologically-mediated inflammatory disorder is myasthenia gravis, Guillain Barré syndrome, and other inflammatory diseases of the nervous system; psoriasis, pemphigus vulgaris and other diseases of the skin; systemic lupus erythematosus, glomerulonephritis and other diseases affecting the kidneys; atherosclerosis and other inflammations of the blood vessels, autoimmune hepatitis, inflammatory bowel diseases, pancreatitis, and other conditions of the gastrointestinal system; type 1 diabetes mellitus, thyroiditis, and other diseases of the endocrine system.

41. A method for the treatment of inflammation, particularly immunologically-mediated inflammation, which comprises administering to a patient in need an effective amount of an immunomodulator selected from: (a) a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty alcohol or an ester thereof with a $C_1 - C_6$ alkanolic acid; (b) a monoester of a $C_2 - C_8$ alkanediol or of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid; and (c) a diester of glycerol with a saturated or cis-unsaturated $C_{10} - C_{20}$ fatty acid.

42. A method according to claim 41, wherein said immunomodulator is a saturated $C_{10} - C_{12}$ fatty alcohol.

43. The method according to claim 42, wherein said saturated $C_{10} - C_{20}$ fatty alcohol is selected from decyl alcohol, lauryl alcohol, myristyl alcohol, cetyl alcohol and stearyl alcohol.

44. The method according to claim 41, wherein said immunomodulator is a cis-unsaturated C₁₆-C₁₈ fatty alcohol.

5 45. The method according to claim 44, wherein the cis-unsaturated C₁₆-C₁₈ fatty alcohol is selected from oleyl alcohol, linoleyl alcohol, γ -linolenyl alcohol and linolenyl alcohol.

46. The method according to claim 41, wherein the immunomodulator is an ester of a
10 saturated or cis-unsaturated C₁₀ - C₂₀ fatty alcohol with a C₁ - C₆ alkanolic acid.

47. The method according to claim 41, wherein said immunomodulator is a monoester of a saturated or cis-unsaturated C₁₀ - C₂₀ fatty acid with a C₂ - C₈ alkanediol.

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48. The method according to claim 47, wherein said alkanediol is selected from 1,2-ethylene glycol, 1,3-propanediol and 1,4-butanediol.

49. The method according to claim 41, wherein said immunomodulator is a
20 monoester of glycerol with a saturated or cis-unsaturated C₁₀-C₂₀ fatty acid.

50. The method according to claim 41, wherein said immunomodulator is a diester of glycerol with a saturated or cis-unsaturated C₁₀-C₂₀ fatty acid.

25 51. The method according to any one of claims 41 and 47 to 50, wherein said fatty acid is a saturated C₁₀-C₂₀ fatty acid.

52. The method according to claim 51, wherein said saturated C₁₀-C₂₀ fatty acid is selected from capric acid, lauric acid, myristic acid, palmitic acid, stearic acid and
30 arachidic acid.

53. The method according to any one of claims 41 and 47 to 50, wherein said fatty acid is a cis-unsaturated C₁₀-C₂₀ fatty acid.

5 54. The method according to claim 53, wherein said cis-unsaturated C₁₀-C₂₀ fatty acid is selected from palmitoleic acid, oleic acid, cis-vaccenic acid, linoleic acid, γ -linolenic acid, linolenic acid, and arachidonic acid.

10 55. The method according to claim 54, wherein said immunomodulator is glyceryl monooleate.

56. The method according to claim 54, wherein said immunomodulator is glyceryl dioleate.

15 57. A method according to any one of claims 41 to 56 for the treatment of immunologically-mediated inflammatory disorders selected from an autoimmune disease, severe allergies, asthma, graft rejection or for the treatment of chronic degenerative diseases such as Alzheimer's disease, and in neuroprotection, organ regeneration, chronic ulcers of the skin, and schizophrenia.

20 58. The method according to claim 57, wherein said autoimmune disease is multiple sclerosis or a human arthritic condition.

25 59. The method according to claim 58, wherein said human arthritic condition is selected from rheumatoid arthritis, reactive arthritis with Reiter's syndrome, ankylosing spondylitis and other inflammations of the joints mediated by the immune system.

30 60. The method according to claim 57, wherein said immunologically-mediated inflammatory disorder is myasthenia gravis, Guillain Barré syndrome, and other

inflammatory diseases of the nervous system; psoriasis, pemphigus vulgaris and other diseases of the skin; systemic lupus erythematosus, glomerulonephritis and other diseases affecting the kidneys; atherosclerosis and other inflammations of the blood vessels, autoimmune hepatitis, inflammatory bowel diseases, pancreatitis, and
5 other conditions of the gastrointestinal system; type 1 diabetes mellitus, thyroiditis, and other diseases of the endocrine system.

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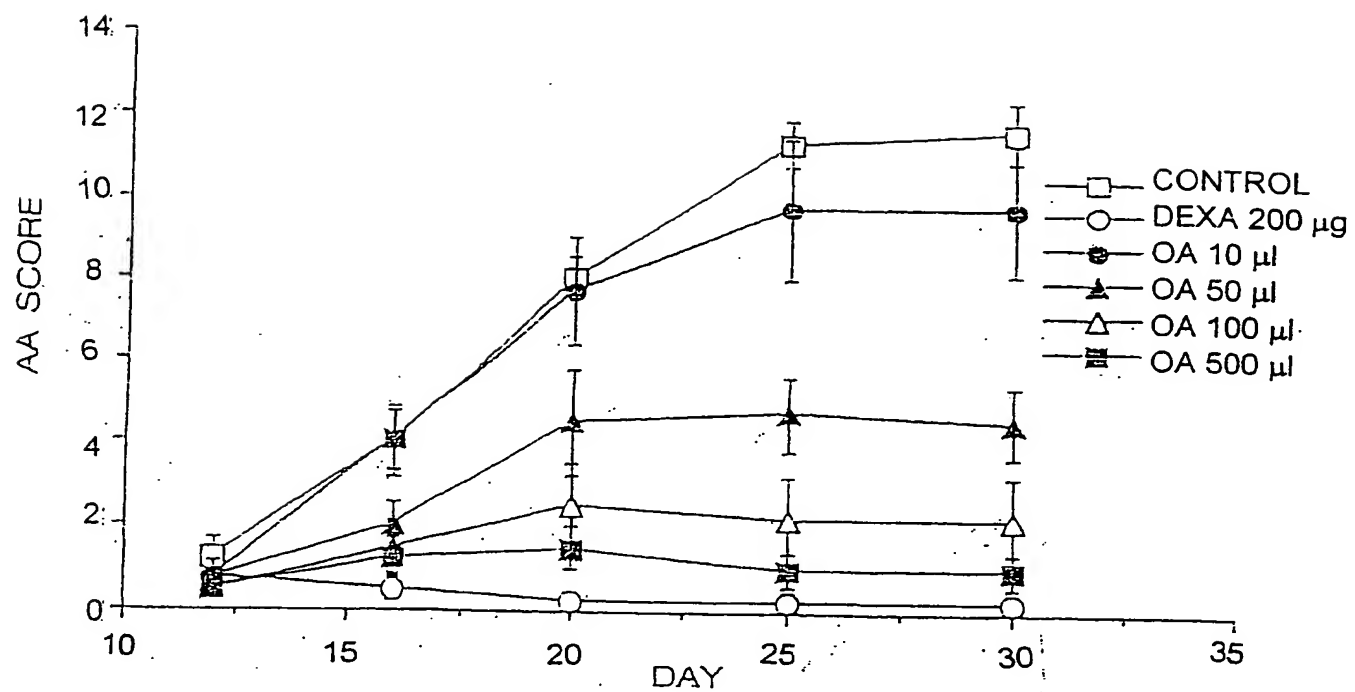


Fig. 1

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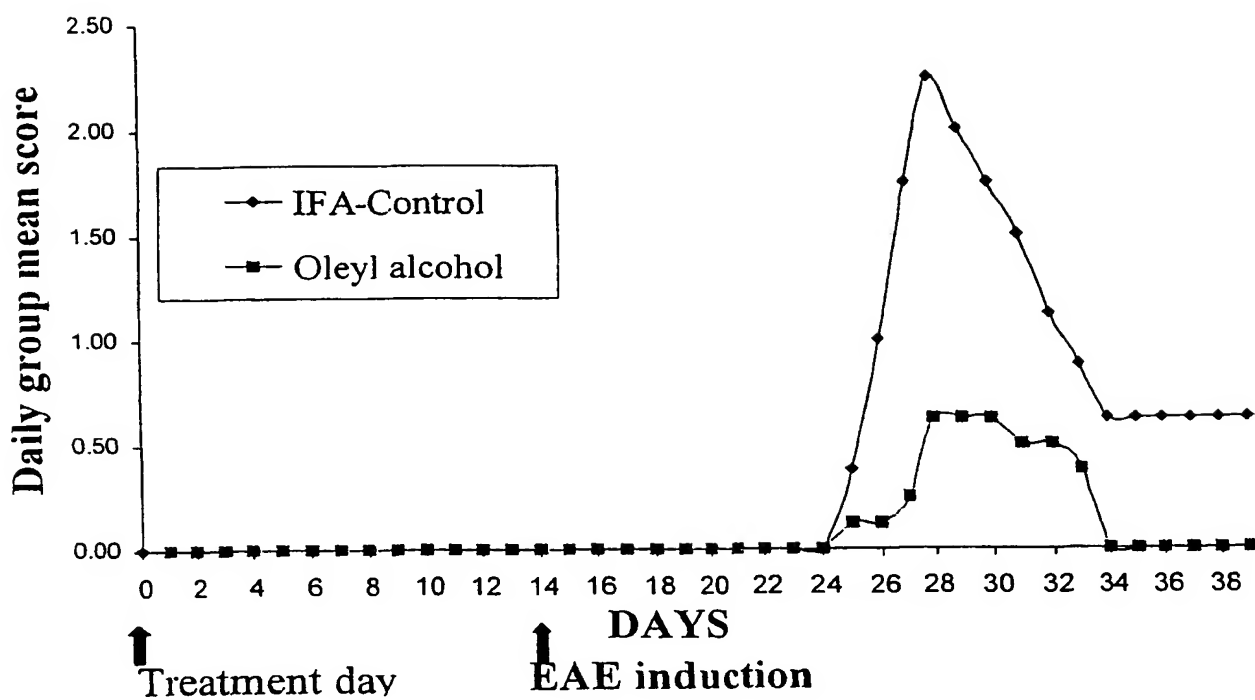


Fig. 2

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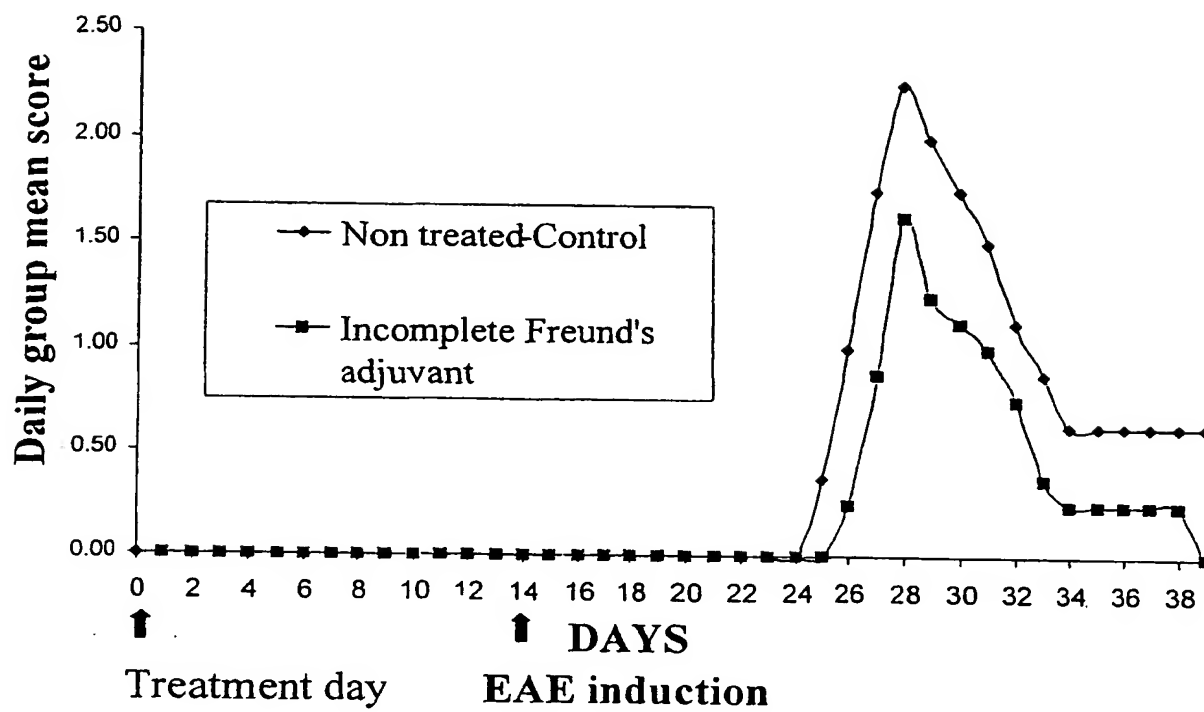


Fig. 3

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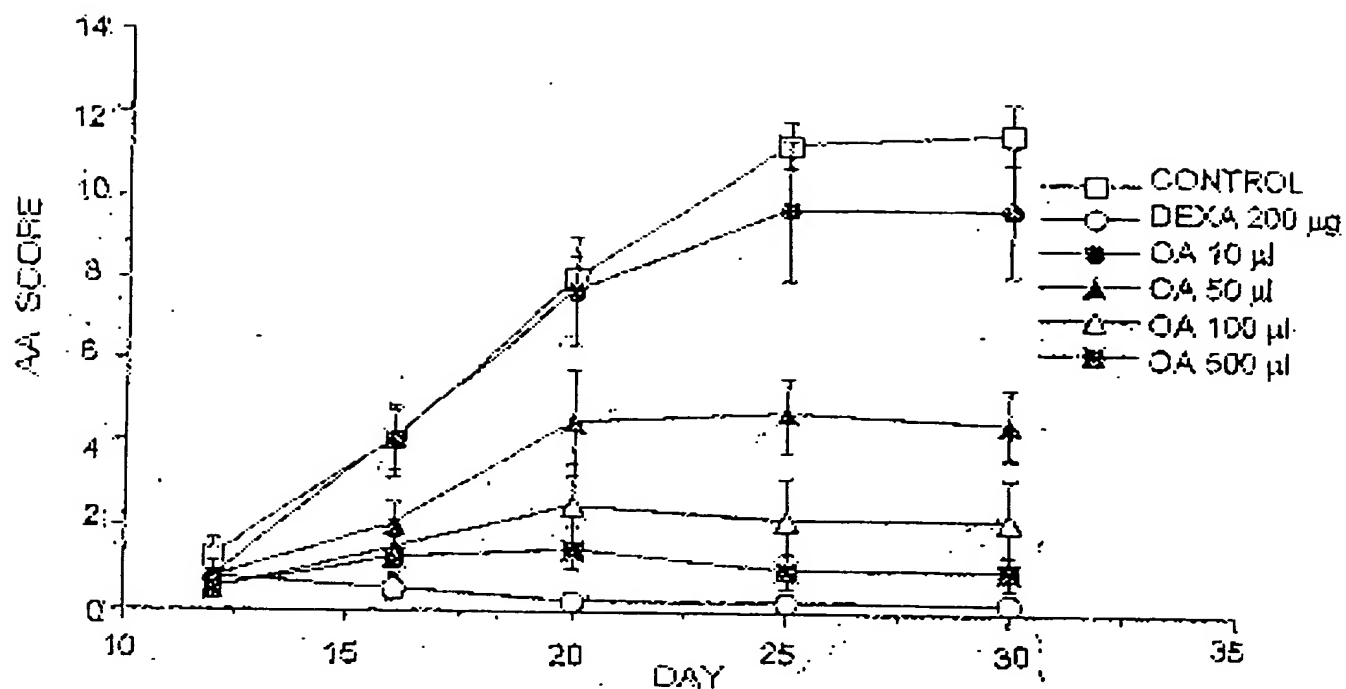


Fig. 1

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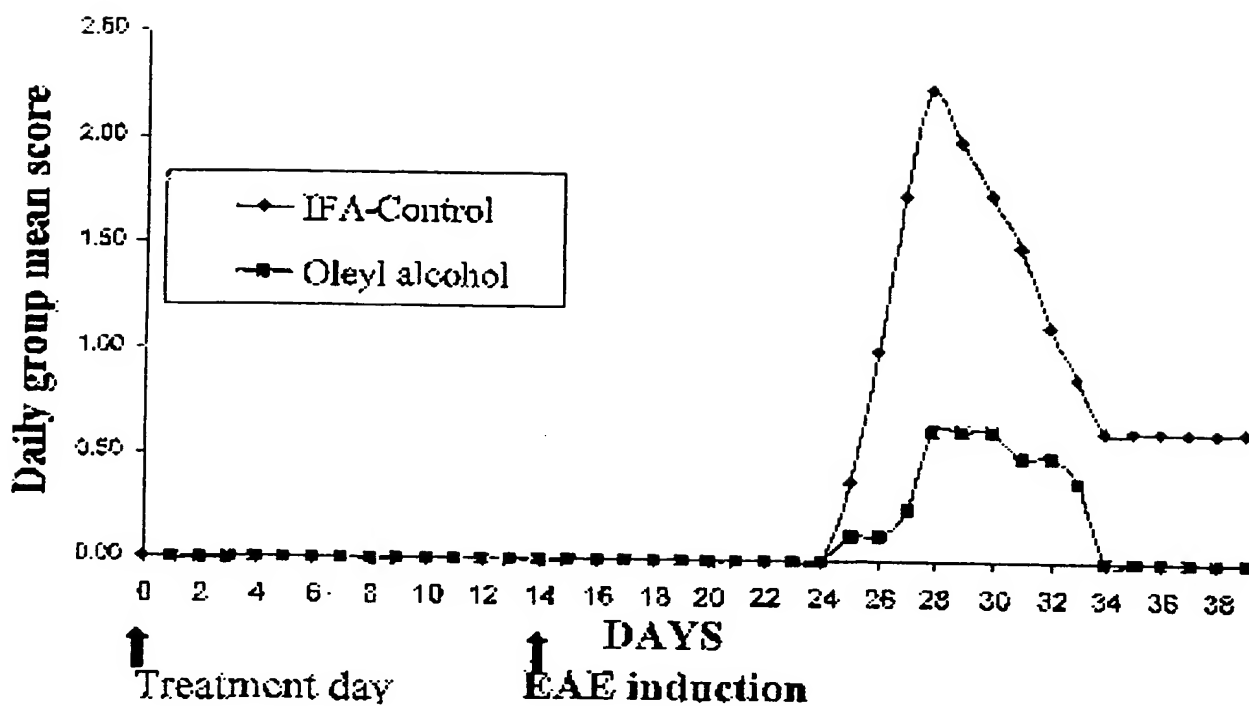


Fig. 2

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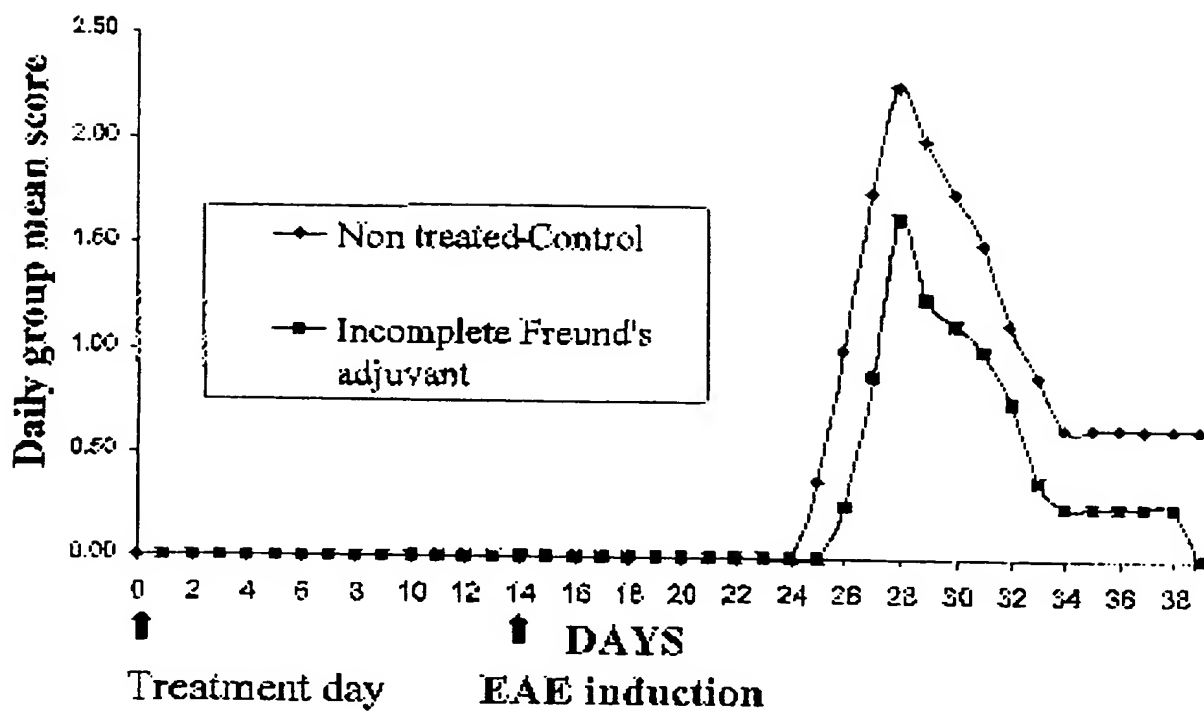


Fig. 3